THE INCIDENCE AND DISTRIBUTION OF *CLOSTRIDIUM BOTULINUM* IN SOILS OF ILLINOIS

BY

MORRIS THOMPSON JONES

A.B., University of Illinois, 1940

M.S., University of Illinois, 1941

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN
BACTERIOLOGY IN THE GRADUATE SCHOOL OF
THE UNIVERSITY OF ILLINOIS, 1944

URBANA, ILLINOIS

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Botulism was raised from a place of obscurity to a place of prominence on account of outbreaks resulting from the use of unsafe canning methods during the first World War. Increase in number of recognized cases raised many questions, among which the source of the etiological agent was of great importance from the public health standpoint of both home and commercial canning. Epidemiological data and characteristics of the organism incriminated soil, and suggested the need for investigation of the incidence of *Clostridium botulinum* in soil. Although limited investigations of this subject have been made in Illinois, no one has carried out a comprehensive investigation. The economy of a second World War, with its attendant shortage of critical materials for production of equipment with which to follow safe methods of canning, has re-emphasized the need for such an investigation. It is hoped that data obtained through this survey will give further basis for evaluating and correlating the present information on botulism which is so closely related to the problem of food preservation.

Among the earlier investigations of specimens from Illinois was that of Meyer and Dubovsky who examined 14 soil samples and 25 other specimens consisting of plant parts sent to them by several Illinois canning companies. Twelve and eight-tenths per cent of these specimens produced toxic cultures of which two were determined to be *Clostridium botulinum* type B. A more extensive investigation of Illinois soils was reported by Tanner and Dack the same year. These investigators examined 56 soil samples from Illinois; 33 of which were collected from various places around Urbana and 22 were obtained from other parts of the state. Ten, or 17.8 per cent, of the cultures were toxic and those typed were also found to contain the toxin of *Clostridium botulinum* type B. Graham and Boughton recovered *Clostridium botulinum* type C from soils of poultry yards in Macon, Cumberland, Christian, and Brown counties, where symptoms of botulism occurred in chickens and ducks and from a barnyard in Green County where horses died of a disease characterized by general paresis. Bengston was also successful in recovering *Clostridium botulinum* type C from soil samples obtained in Illinois where limberneck in poultry was prevalent.

In this investigation soil samples were obtained from different sources. Sterile, cotton-stoppered test tubes were used for collection of soil samples. With modifications, the procedure followed in preparing samples for culture was essentially that of transferring 10 g. of each soil sample to test tubes sterilized after addition of 30 ml. of distilled water and 0.5 g. of precipitated calcium carbonate. Test tubes were fitted with sterile rubber stoppers, shaken vigorously and heated in a water bath for 30 minutes at 80° C. Subsequent rapid cooling was accomplished by means of either cold water or air. The sample was again suspended by shaking before transferring it to a flask of enrichment medium consisting of liver infusion with liver particles. After incubation for 10 days at 37°C. each culture was tested for toxin production by feeding one ml. of the enrichment culture to a guinea pig.

The first 100 soil samples received were examined in a more thorough manner than those just described. Enrichment cultures of each sample were prepared in duplicate. The soil inoculum of one culture was heated as described above but the inoculum of the other culture was not heated. After incubation for 10 days at 37°C. these enrichment cultures were tested for toxicity by feeding one ml. of each culture to guinea pigs. Shake cultures were prepared from both enrichment cultures and five colonies, supposedly typical of *Clostridium botulinum*, were picked from each shake culture and placed in a thioglycollate medium. After incubation at 37°C. for the same period of time, these latter cultures were also tested for toxin production by feeding one ml. of each culture to guinea pigs.

A total of 604 soil samples were obtained and examined from 88 counties in Illinois. Forty-four, or 7.2 per cent, of the samples produced toxic cultures. One or more toxin-producing soil samples was obtained from the following counties: Champaign, Christian, Clark, Clay, Clinton, Cook, DeWitt, Douglas, Edwards, Jefferson, Jo Daviess, Johnson, Kankakee, Macon, Macoupin, Madison, Monroe, Montgomery, Piatt, Pope, Rock Island, St. Clair, Sangamon, Wabash, Wayne, and White. The toxin of one culture which has been typed to date was identified as that of *Clostridium botulinum* type B.

The geographical distribution of toxin-producing soil samples indicates that the majority of such samples were obtained from the southern half of the state. Omitting St. Clair County, which will be considered individually, 12.1 per cent of 279 samples from

the southern half of the state produced toxic cultures. Toxin-producing samples were obtained from 36.5 per cent of 52 counties in the southern part of the state. In contrast to these figures are those for the other half of the state where 3.65 per cent of 274 samples examined produced toxic cultures and were obtained from 14 per cent of 50 counties in the northern portion of the state. St. Clair County offered an opportunity for making a more intensive survey of a limited area than could be made for all portions of the state. Fifty-one soil samples were examined from this county and 11.8 per cent of them produced toxic cultures.

In general, soils which produced the greatest number of toxic cultures appeared to be the agriculturally less productive yellow soils of southern Illinois. The possibility that physical-chemical characteristics and microbiological relations of the rich, black soils of the Illinois Cornbelt preclude the detection of *Clostridium botulinum* by the techniques used should not be overlooked. Limitations of the techniques available for the detection of *Clostridium botulinum* in soil are exceedingly great, and it must be recognized that results obtained can only be considered as indicating a minimal distribution. This statement is corroborated by experimental data obtained in this investigation which show that attempts at actual isolation of *Clostridium botulinum* reveal toxin-producing samples which enrichment cultures do not.

Although these figures for the occurrence of *Clostridium botulinum* in soils of Illinois may appear low they are nevertheless significant. Rather than depreciate the hazard of botulism in Illinois, these figures serve to confirm further that botulism is a possibility in Illinois and that the danger is even more widespread than previous investigators have shown.

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